The Response

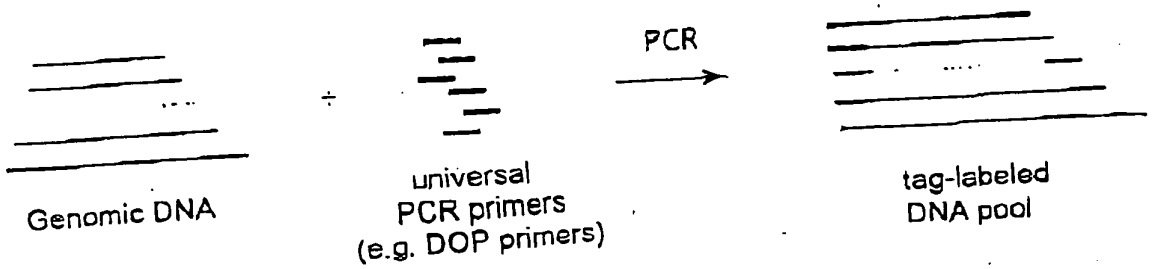
Rejections under 35 U.S.C. § 112, First Paragraph: Enabling and Written Description

Claims 1-7 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabling for the method as presently claimed. Claims 1-5 and 8 remain rejected under 35 U.S.C. §112, first paragraph, as allegedly not satisfying the written description requirement. Claim 8 is canceled. The rejections of the remaining claims are overcome in parts in view of the amendments and traversed in parts.

Applicants are submitting the following explanation to Claim 1.

(a) DNA is isolated from normal cells, i.e., cells having no known numerical changes in the DNAs, and amplified by means of a PCR method using tag primers. Tag-labeled DNA pool is obtained (see Fig. 1.1.).

(Fig. 1.1.) Preparation of a representative tag-labeled DNA pool from genomic DNA of normal cells by means of a universal PCR method.

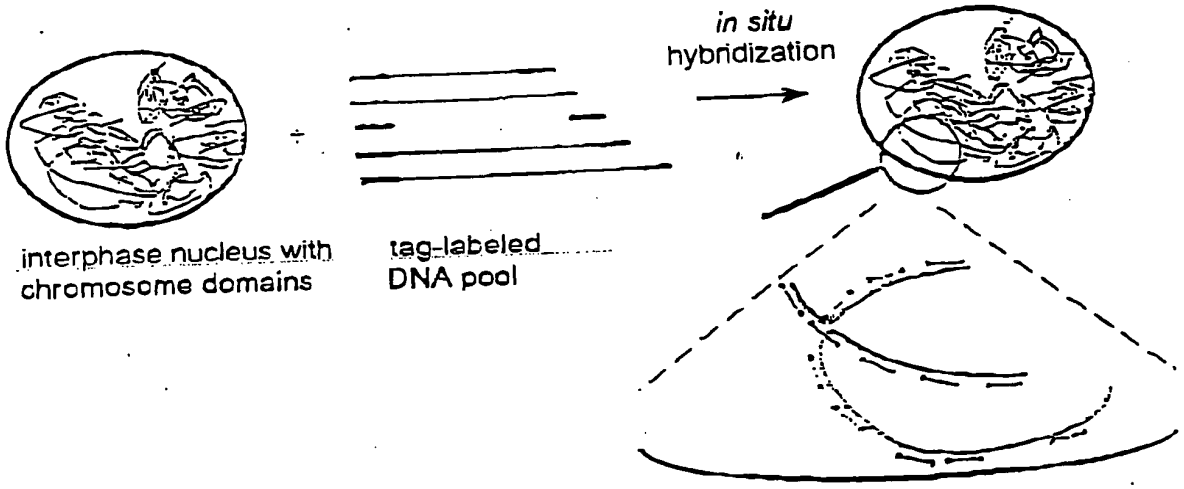


The amplified DNAs are then labeled with a first label, such as digoxygenin-UTP (see page 6, lines 5-7).

(b) Cells under study, e.g., interphase nuclei of tumor cells, are hybridized *in*

situ with the tag-labeled DNA pool of (a) (see Fig. 1.2.).

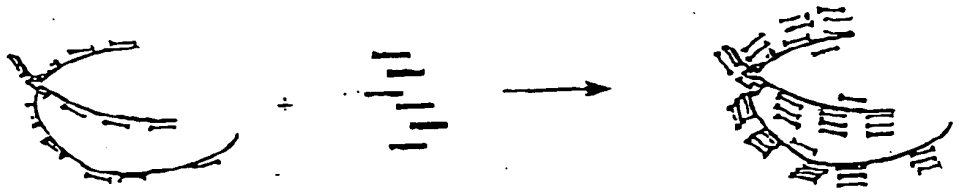
(Fig. 1.2.) *In situ* hybridization of interphase nuclei with tag-labeled DNA pool.



(c) DNAs from the in situ hybridized cells of (b) are amplified by means of a PCR method using the tag primers of (a). Amplifications at two different regions of the DNAs from the in situ hybridized cells are obtained:

1. At regions comprising the tag labeled DNA pool of (a).
2. At regions other than the regions of 1, where additional sequences, i.e., DNA sequences of numerical changes, are located which can be amplified by the tag primers of (a) (see Fig. 1.3.).

(Fig. 1.3.) Universal PCR of the isolated interphase nuclei with identical primers (see step a).



The amplified DNAs are then labeled with a second label that is different from the first label, e.g., a biotin-label. (See page 12, lines 1-3 (in *Human Chromosomes*, page 195)).

(d) The DNAs of (a) and (c) are co-hybridized with metaphase chromosoms breads from normal cells under suppression hybridization conditions.

(e) Numerical changes in the amplified DNA of (c) are identified.

The steps (d) and (e) represent a common comparative genomic hybridization (CGH) analysis (see page 11, lines 23-26). A copy of page 195 of *Human Chromosomes* is submitted herewith to demonstrate a schematic illustration of CGH.

In summary, the present method is a combination of steps (a)-(c), which prepare tag-labeled DNA pools from normal cells and test cells (tagged genomic hybridization, TGH) and steps (d)-(e), which represent a common CGH analysis. The results of Figure 2 show that the combination of TGH and CGH processes is (i) more sensitive in detecting the over-representation of chromosomes (89% - 94% versus 38% - 44%), and (ii) more specific in lowering the false-positive findings, compared with the process without TGH (see page 12, lines 3-8).

Applicant believes that Claim 1 as amended, fulfils the enabling and written description requirements of 35 U.S.C. § 112, first paragraph. The Examiner is requested to withdraw the rejection of Claims 1-7.

CONCLUSION

It is now believed that the claims are in condition for allowance and advancement as such is earnestly requested. Should any questions arise in connection with this submission which may be resolved by a telephonic interview, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

Date: February 21, 2002

Albert P. Halluin (Reg. No. 25,227)
Viola T. Kung (Reg. No. 41,131)

Enclosure: Page 195 of Human Chromosomes

HOWREY SIMON ARNOLD & WHITE, LLP
301 Ravenswood Avenue
Box No. 34
Menlo Park, CA 94025
(650) 463-8109

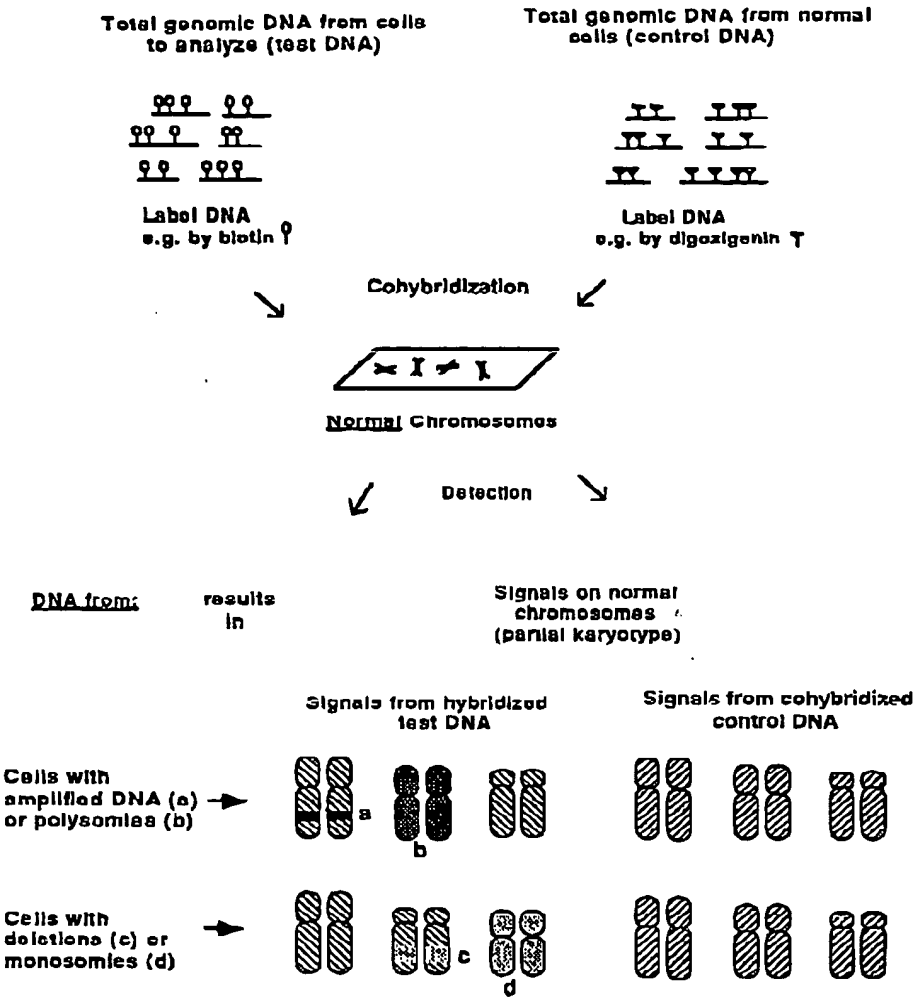


Figure 6.8.
Schematic illustration of comparative genomic hybridization (CGH). Labeled genomic DNA from tissue samples to be analyzed (test DNA) and differently labeled DNA derived from normal tissue (control DNA) are hybridized simultaneously to normal metaphase chromosomes. As a result, a general background staining of all chromosomes is obtained (dashed lines). Chromosomal sequences present in additional copies within the test DNA (i.e., due to amplifications or polysomies) result in a higher staining at the corresponding chromosomal target sequences (indicated in black), as compared with the control DNA. Losses of chromosomal sequences (i.e., due to deletions or monosomies) result in a weaker staining of the corresponding target chromosomes (indicated by a lighter staining).

monly detect hybridized test and control DNA with FITC and rhodamine, respectively. However, the fluorochromes can also be used conversely without hampering the quality of the analysis.

Diagnostic variations of signal intensities in CGH can only be obtained when the signal portion of the ubiquitously occurring interspersed repetitive sequences (IRS) within DNA probes is efficiently suppressed. Suppression has to be as complete as possible in order to allow an accurate measurement of the fluorescence intensities. Standard in situ suppression hybridization protocols for genomic DNA probes (Landegent et al., 1987; Lichter et al., 1988; Pinkel et al., 1988), as generally used for gene mapping or in clinical diagnostics, allow for some background staining through IRS. In contrast, CGH requires a rather complete suppression of IRS signals. This is achieved by using large amounts of human Cot1 DNA, representing the fast associating

VERSION WITH MARKING TO SHOW CHANGES MADE

1. (Three Times Amended) A process for detecting chromosomal overrepresentation in cells, comprising the following steps:

(a) isolating DNAs from cells which have no known numerical changes in their DNAs, [and] amplifying the DNAs by means of a PCR method using tag primers, and labeling the amplified DNAs with a first label;

(b) hybridizing cells under study *in situ* with the amplified DNAs from (a);

(c) amplifying DNAs from the *in situ* hybridized cells from (b) by means of a PCR method using the tag primers from (a), and labeling the amplified DNAs of (c) with a second label that is different from the first label;

(d) cohybridizing the labeled DNAs from (a) and (c) to metaphase chromosome spreads from normal cells under suppression hybridization conditions; and

(e) identifying numerical changes in the amplified DNAs from (c).

Cancel Claim 8.